Accession: 0148604 Year: 98 Project Number: 1265-32000-045-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: EPIDEMIOLOGY & CONTROL OF TOXOPLASMA, TRICHINELLA & RELATED PARASITES IN DOMESTIC ANIMALS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Toxoplasma gondii, Neospora caninum, Sarcocystis neurona, and Trichinella spiralis are parasites that can cause serious disease in humans and/or animals. The first three are morphologically related single-celled parasites, whereas T. spiralis is a nematode. The major issue with respect to T. gondii and T. spiralis is food safety, primarily related to pork products. Consumers have long been aware of the potential presence of worms (T. spiralis) in undercooked pork and they are becoming increasingly aware of the fact that T. gondii is found in pork and other types of meat. Both parasites pose a risk to consumers who eat raw/undercooked or otherwise improperly prepared meat. Neosporosis, caused by Neospora caninum, is a major cause of abortion in cattle, and it also causes paralysis in other animals. Sarcocystis neurona is the most important cause of neurologic illness in horses in the United States. The objectives of research conducted on these parasites includes reducing or eliminating risk of human exposure from contaminated pork or other meats products (in the case of T. gondii and T. spiralis), preventing transmission of Neospora to dairy and beef cattle, and preventing transmission of S. neurona from opossums to horses. For all four parasites, understanding their biology and epidemiology is the major area of research.

2. How serious is the problem? Why does it matter?

A 1990 survey estimated the annual cost of food borne congenital toxoplasmosis in humans to be about 2.6 billion dollars in the U.S. A conservative estimate for losses associated with Neospora-induced abortion in dairy cattle in California alone is approximately 35 million dollars annually, not accounting for the cost of culling the cows, re-breeding the cows, and loss of milk production. Sarcocystis neurona is the most important cause of neurologic disease in horses. Trichinella

spiralis is the #1 consumer concern with respect to the safety of pork products. The U.S. requires extensive processing of all ready-to-eat pork products due to this parasite. Trading partners require testing of pork products prior to shipment, and the image of U.S. pork with respect to Trichinella infection impacts accessability to foreign markets.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research conducted on this project supports National Program objectives in Animal Health and Food Safety (National Program 103, Animal Diseases and National Program 108, Food Safety). Objectives of research on the zoonotic pathogens Toxoplasma and Trichinella focus on detection, prevalence, transmission, and epidemiology. The goal of this research is to establish farm management systems (pre-harvest interventions) which limit or eliminate risk of infection in food animals. Neospora and Sarcocystis neurona are newly recognized and emerging pathogens. Basic information is needed on the biology and epidemiology of these parasites so that control programs based on management or vaccine strategies may be designed.

4. What was your most significant accomplishment this past year?

Dogs were discovered to be the definitive (reservoir) host for Neospora caninum. A resistant stage of the parasite (called the oocyst) was found in the feces of dogs fed infected tissues. Until now, vertical transmission (transfer from cow to fetus) was the only known mode of transmission of Neospora caninum, and Neospora-induced abortion storms remained unexplained until the oocyst was discovered. This finding is the key to control of neosporosis.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Methods were developed for the accurate detection of Toxoplasma gondii infection in pigs, including ongoing studies on the use of recombinant antigens in an ELISA test. Information on the prevalence of Toxoplasma in pigs raised under various management systems was collected. Risk factors associated with acquisition of T. gondii in pigs raised under different management systems were studied and cats and mice were found to be the main source of T. gondii infection in pigs. Studies were initiated to identify point sources of oocyst contamination on farms where high prevalence was found. Methods (cooking, freezing, irradiation) were developed to kill Toxoplasma parasites in meat and meat products. An audit and verification system was developed to allow pigs to be certified free from Trichinella infection at the farm. The audit is based on evaluation of management practices which are known to limit risk of exposure of pigs to infection. Periodic testing of certified pigs will be conducted using second generation diagnostic

tests developed and commercialized through ARS research. Methods used for the inspection of pigs and horses for Trichinella at slaughter were evaluated and recommendations made to improve existing regulations. An export market inspection program was established and maintained in cooperation with the Agricultural Marketing Service. Neospora caninum, a major cause of abortion in cattle, was identified, named, diagnostic tests developed and animals models were developed to screen drugs and

Accession: 0148604 Year: 98 Project Number: 1265-32000-045-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

vaccines. Sarcocystis neurona, the cause of fatal encephalomyelitis in horses was named, cultured, diagnostic tests developed, and its source of infection (the opossum) was identified.

6. What do you expect to accomplish during the next year?

We will continue studies on the biology and epidemiology of N. caninum and S. neurona, including immunity to shedding of N. caninum oocysts in dogs, survival of N. caninum oocysts in the environment, and elucidation of the life cycle of N. caninum in the intestines of dogs. The life cycle of S. neurona remains unknown and the search for the source of S. neurona infection in the opossum will continue. A new S. neurona-like parasite was recently discovered in our laboratory and studies on its life cycle and epidemiology will continue. On farm epidemiology of T. gondii on swine farms in New England states will continue, as will development of rapid and sensitive tests for detection of infection in pigs. Management systems which reduce the risk of exposure to Toxoplasma infection will be identified and monitored. Sources of oocysts in the environment will studied on infected farms. Research supporting pre-harvest certification programs and inspection programs for export markets for Trichinella will continue.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

ARS research on the detection and transmission of Trichinella spiralis has been used to develop the first pre-harvest certification program for food safety in livestock. Working with the National Pork Producers Council, APHIS and FSIS, a national program was developed and is being implemented, to certify the safety of pigs when they leave the farm for slaughter. Working with private industry, research on improved detection methods for T. spiralis infection has been commercialized into kits for testing tissue fluids during processing. Information generated on risk

factors for toxoplasmosis on swine farms has been communicated to the National Pork Producers Council (NPPC) and these data are being used by the NPPC for education in risk-reduced swine management. Reagents and information supplied to drug companies formed the basis for development and successful marketing of diagnostic kits for neosporosis (for example, Iddex ELISA kit for Neospora). An atlas of protozoan parasites in animal tissues by Gardiner, Fayer and Dubey was revised and a 2nd edition published. This book is used extensively by pathologists and parasitologists worldwide. A review article on the biology of Toxoplasma tissue cysts was published by invitation in Clinical Microbiology

Accession: 0148604 Year: 98 Project Number: 1265-32000-045-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Reviews. There were more than 300 requests for reprints of this paper from scientists all over the world. A Trichinae Fact Sheet was prepared at the request of the American Meat Institute and the National Pork Producers Council and is included in the Pork Facts Handbook provided to producers.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Trichinae, on-farm certification, and Iowa ... Lawrence E. Miller, Iowa Pork Producer, 34(9):5-6.

PUBLICATIONS:

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BARR, B.C., DUBEY, J.P., LINDSAY, D.S., REYNOLDS, J.P. and WELLS, S.J. 1998. Neosporosis: Its prevalence and economic impact. Supplement to Comp. Cont. Ed. Prac. Vet. 20:4-16.

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DAVIES, P.R., MORROW, M.E., PATTON, S., GAMBLE, H.R. and DEEN, J. 1998. Seroprevalence of ... swine raised in different production systems in North Carolina, USA. Prev. Vet. Med. 36:67-76.

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DEVADA, K., ANANDAN, R. and DUBEY, J.P. 1998. Serologic prevalence of Toxoplasma gondii in chickens in Madras, India. J. Parasitol. 4:621-622.

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DUBEY, J.P. 1998. Refinement of pepsin digestion method for isolation of Toxoplasma gondii from infected tissues. Vet. Parasitol. 74:75-77.

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DUBEY, J.P., ABBITT, B., TOPPER, M.J. and EDWARDS, J.F. 1998. Hydrocephalus associated with Neospora caninium-infection in an aborted bovine fetus. J. Comp. Pathol. 118:169-172.

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DUBEY, J.P., LUNNEY, J.K., SHEN, S.K. and KWOK, O.C.H. 1998. Immunity to toxoplasmosis in pigs fed irradiated Toxoplasma gondii oocysts. J. Parasitol. 84:749-752.

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DUBEY, J.P., DOROUGH, K.R., JENKINS, M.C., LIDDELL, S., SPEER, C.A., KWOK, O.C.H. and SHEN, S.K. 1998. Canine neosporosis: ... Neospora caninum in mice and cell culture. Int. J. Parasitol. 28:1293-1304.

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DUBEY, J.P., ROMAND, S., HILALI, M., KWOK, O.C.H. and THULLIEZ, P. 1998. Seroprevalence of antibodies to N. caninium and T. gondii in water buffaloes (Bubalus bubalis) from Egypt. Int. J. Parasitol. 28:527-529.

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MCALLISTER, M.M., DUBEY, J.P., LINDSAY, D.S., JOLLEY, W.R., WILLS, R.A. and MCGUIRE, A.M. 1998. Dogs are definitive hosts of Neospora caninum. Int. J. Parasitol. 28:1473-1478.

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MONDRAGON, R., HOWE, D.K., DUBEY, J.P. and SIBLEY, L.D. 1998. Genotypic analysis of T. gondii isolates from pigs. J. Parasitol. 84:639-641.

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Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400168 Year: 98 Project Number: 1265-32000-045-02 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: DEVELOPMENT OF AN ELISA TEST FOR TOXOPLASMOSIS IN PIGS

Period Covered From: 01/98 To: 09/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Toxoplasma gondii is a protozoan parasite that causes serious disease in humans and animals. The major agricultural issue with respect to T. gondii is food safety, primarily related to pork products. Consumers are becoming increasingly aware of the fact that T. gondii is found in pork and other types of meat and poses a risk to consumers who eat raw/undercooked or otherwise improperly prepared meat. The objective of research conducted on T. gondii is to reduce or eliminate risk of human exposure from contaminated pork or other meats products. To accomplish this objective adequate tools for detection of infected pigs are needed. This research was conducted as part of a CRADA with Safe-Path Laboratories to develop an ELISA for detection of T. gondii infection in pigs.

2. How serious is the problem? Why does it matter?

A 1990 survey estimated the annual cost of food borne congenital toxoplasmosis in humans to be about 2.6 billion dollars in the U.S. While some human infection result from environmental contamination with T. gondii oocysts, a portion of infections results from ingestion of infected meat. Prevalence in pigs ranges as high as 50% in parts of the U.S. The pork industry is aware of this problem and is interested in solutions based on improved management systems or other methods to reduce incidence in pigs.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research conducted on this project supports National Program objectives in Animal Health and Food Safety (National Program 103, Animal Diseases and National Program 108, Food Safety). Objectives of research on the zoonotic pathogens such as Toxoplasma focus on detection, prevalence, transmission, and epidemiology. The overall goal of this research is to establish farm management systems (pre-harvest interventions) which limit or eliminate risk of infection in food animals. The intermediate goal of this project is to develop adequate tools for detection of infection.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400168 Year: 98 Project Number: 1265-32000-045-02 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

4. What was your most significant accomplishment this past year?

An ELISA test based on a tachyzoite antigen extract was used to detect infection in pigs using a rapid (10 minute) format. The ELISA performed as well as the modified agglutination test which is widely used for detection of Toxoplasma infection.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Methods were developed for the accurate detection of Toxoplasma gondii infection in pigs, including ongoing studies on the use of recombinant antigens in an ELISA test. The ELISA test was further modified to run using juices extracted from meat samples. Additional test format changes are being evaluated, including a lateral flow system, which can be performed using multiple parasite antigens.

6. What do you expect to accomplish during the next year?

This CRADA has terminated (30 October, 1998), but research on detection of T. gondii infection in pigs using recombinant antigens continues.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

No further work will be conducted on this expired CRADA.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400414 Year: 98 Project Number: 1265-32000-045-03 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: DEVELOPMENT OF A MOUSE MODEL FOR NEOSPOROSIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Neosporosis is a protozoan disease of domestic animals. In cattle, it is a major cause of reproductive failure in the dairy and beef industries. Vaccination of cows against the parasite is being attempted by our laboratory to prevent infection in cows and transmission of the parasite to the fetus. Our research effort is directed at developing a congenital infection model in mice for testing vaccines against the disease. If these vaccines prove to be effective against infection, then similar immunization protocols will be attempted in cows.

2. How serious is the problem? Why does it matter?

Neosporosis has been found to be responsible for 25-50% of all abortions in dairy cows in the U.S. resulting in greater than $100 million in losses to the dairy industry each year. The disease is widespread and appears to be endemic in most dairy herds in the U.S.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This disease is directly related to the development of control measures for preventing parasitic diseases in domestic animals using alternative approaches to drug treatment (National Program 103, Animal Diseases).

4. What was your most significant accomplishment this past year?

The most significant accomplishment in 1998 was attainment of complete

immunity against congenital infection in mice by immunization with a preparation of Neospora caninum antigens.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

The major accomplishments over the life of this project include the development of a mouse model of congenital infection that appears to replicate infections in pregnant cows and the demonstration of protective immunity elicited by vaccination with a preparation of

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400414 Year: 98 Project Number: 1265-32000-045-03 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Neospora caninum antigens.

6. What do you expect to accomplish during the next year?

Anticipated accomplishments for 1999 will be the cloning and expression of DNA encoding "protective" Neospora caninum antigens for eventual testing in the mouse model.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The vaccine technology has not been transferred to industry because studies are needed in cows to show application to the dairy industry. Once recombinant antigen clones are identified and show protective immunity in the mouse model, testing in dairy cows will commence. It may be 2-3 years before the technology is transferred to a biotechnology company for preparation of commercial vaccine.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:
01.
LIDDELL, S., LALLY, N.C., JENKINS, M.C. and DUBEY, J.P. 1998. Isolation of the cDNA encoding a dense granule associated antigen (NCDG2) of N. caninum. Mol. Biochem. Parasitol. 93:153-158.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400762 Year: 98 Project Number: 1265-32000-045-04 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: DEVELOPMENT OF DIAGNOSTIC AND INFECTIVITY ASSAYS AND IRRADIATION PROCEDURES FOR CYCLOSPORA

Period Covered From: 01/98 To: 09/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Cyclospora cayetanensis is a single celled parasite that causes diarrhea in humans. Fecal-oral transmission is the only recognized route of infection and humans are the only known hosts. Several outbreaks of cyclosporosis have occurred in humans in the U.S. which have been epidemiologically linked to eating uncooked fruits and salads, particularly raspberries imported from Guatemala. There are no sensitive methods to detect Cyclospora parasites on fruit or to kill the parasite in uncooked fruit. There is no animal model for cyclosporosis. To address these research needs, a CRADA was developed with the University of Arizona.

2. How serious is the problem? Why does it matter?

Cyclospora is a serious disease of humans and outbreaks occur sporadically. Because the parasite cannot be propagated in cell culture, it is difficult to study methods for detection or inactivation.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research addresses objectives in Animal Health (National Program 103) and Food Safety (National Program 108) by defining the transmission pattern of an emerging disease and controlling a microbiol pathogen transmitted by fruits and vegetables.

4. What was your most significant accomplishment this past year?

A coccidian parasite model was developed to study killing of parasites on contaminated fruit using irradiation. Toxoplasma gondii, a coccidian parasite similar to Cyclospora, was chosen for this project because there is no method to test the infectivity of Cyclospora oocysts. A low level of irradiation (less than 0.5 kGy) was found to be effective in killing T. gondii oocysts on deliberately contaminated strawberries. The results suggest that irradiation may be effective in killing Cyclospora on contaminated fruit. Results of irradiation experiments using Cyclospora oocysts from human feces were not conclusive because

Accession: 0400762 Year: 98 Project Number: 1265-32000-045-04 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

microscopic examination was not sensitive and no method is available to test for infectivity of Cyclospora oocysts.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Methods to develop a cell culture system for infectivity of Cyclospora were unsuccessful.

6. What do you expect to accomplish during the next year?

This Project has been terminated.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:
01.
DUBEY, J.P., THAYER, S.W., SPEER, C.A. and SHEN, S.K. 1998. Effect of gamma irradiation on unsporulated and sporulated Toxoplasma gondii oocysts. Int. J. Parasitol. 28:369-375.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400817 Year: 98 Project Number: 1265-32000-045-05 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: VACCINES FOR NEOSPORA AND TOXOPLASMA

Period Covered From: 01/98 To: 11/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Neospora caninum and Toxoplasma gondii are closely related protozoan parasites that cause abortion in livestock. Neospora caninum is a major cause of abortion in dairy cattle, particularly in California. There are no vaccines to prevent neosporosis and toxoplasmosis abortion. A CRADA was established with Bayer Inc. to develop vaccines. The ARS obligation was to supply strains of Neospora and Toxoplasma to Bayer and Bayer compensated ARS for the strains supplied.

2. How serious is the problem? Why does it matter?

Neosporosis induced abortion in dairy cattle in California alone is estimated to cause $35 million in annual losses to the cattle industry. Toxoplasmosis is a serious debilitating disease of animals and humans. It causes mental retardation and loss of vision in congenitally infected children.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

Neosporosis is an emerging disease and research is required to identify its biology and epidemiology. Control could be facilitated through vaccines. A vaccine for cats is a key requirement for preventing Toxoplasma infection in pigs. This research supports objectives of ARS National Programs in Animal Health (103) and Food Safety (108).

4. What was your most significant accomplishment this past year?

Bayer Inc. has used the strains of N. caninum and T. gondii supplied by ARS to analyze proteins for vaccine development. This work is in progress and no accomplishments can be cited as yet.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

The ARS responsibility in this CRADA was to supply the parasite strains.

6. What do you expect to accomplish during the next year?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400817 Year: 98 Project Number: 1265-32000-045-05 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

This project was terminated on 30 November, 1998.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401528 Year: 98 Project Number: 1265-32000-045-08 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: BIOLOGY AND EPIDEMIOLOGY OF NORTH AMERICAN TRICHINELLA SPECIES

Period Covered From: 03/98 To: 08/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Trichinella spiralis is a nematode parasite that infects a wide variety of species, including humans. The major agricultural issue with respect to T. spiralis is food safety; Trichinella infection results from eating infected meat, primarily pork products. Consumers have long been aware of the potential presence of T. spiralis in pork and the fact that it poses a risk to those who eat raw/undercooked or otherwise improperly prepared meat. The objectives of ARS research conducted on T. spiralis includes reducing or eliminating risk of human exposure from contaminated pork or other meats products. Because there is biological variation in the genus Trichinella, knowledge of risk is predicated on knowledge of the biology of the parasites. Three species of Trichinella are important in North America. In this Cooperative Agreement, ARS and Danish researchers have studied various biological characters of these species of Trichinella.

2. How serious is the problem? Why does it matter?

Trichinella spiralis is the #1 consumer concern with respect to the safety of pork products. The U.S. requires extensive processing of all ready-to-eat pork products. Trading partners require testing of pork products prior to shipment, and the image of U.S. pork with respect to Trichinella infection impacts accessability to foreign markets. As the pork industry moves to develop certification programs which document product safety, it is necessary to understand the risks of pig infection posed by the different species of Trichinella.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research conducted on this project supports National Program objectives in Animal Health and Food Safety (National Program 103, Animal Diseases and National Program 108, Food Safety). Objectives of research on the zoonotic pathogen Trichinella focuses on detection, prevalence, transmission, and epidemiology. The goal of this research is to establish farm management systems (pre-harvest interventions) which limit or eliminate risk of infection in food animals. Basic information

Accession: 0401528 Year: 98 Project Number: 1265-32000-045-08 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

is needed on the biology and epidemiology of this parasite so that control programs based on management may be designed.

4. What was your most significant accomplishment this past year?

The infectivity and freeze resistance of the three major types of North American Trichinella were defined. These results will be valuable in assessing risk to pigs raised under various swine management conditions and in areas where Trichinella is endemic.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Pigs were infected with seven species or types of Trichinella and the course of infection followed. Pigs were tested over 40 weeks for antibody response to evaluate suitability of detection methods. At slaughter, worms were evaluated for reproductive capacity, cyst formation and resistance to freezing. Based on the results, substantial biological differences exist between the species/types tested. Specific recommendations can be made regarding risk associated from the types of Trichinella found in North America. In addition, new knowledge was generated regarding the suitability of freezing to kill parasites in pork and this information will be of value when establishing regulations for meat processing.

6. What do you expect to accomplish during the next year?

This CRADA terminated 31 October, 1998. The remaining work involves writing manuscripts.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The information on biological characters of the North American species/types of Trichinella will be used by the pork industry in designing pre-harvest certification programs. Information on freeze resistance will be used in developing guidelines for preparation of meat products to inactivate trichinae.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401528 Year: 98 Project Number: 1265-32000-045-08 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 40%

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402335 Year: 98 Project Number: 1265-32000-045-10 R
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

Title: DETECTION OF TOXOPLASMA OOCYSTS IN MUNICIPAL WATER

Period Covered From: 09/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Toxoplasmosis is a serious disease of humans and animals. The etiologic agent, Toxoplasma gondii, causes mental retardation and loss of vision in congenitally infected children and abortion in livestock. Humans become infected by ingesting tissue cysts of T. gondii in uncooked meat or by ingesting the resistant stage (oocysts) of the parasite excreted by infected cats. Recently, a major outbreak of toxoplasmosis that occurred in Vancouver, British Columbia, Canada was linked to drinking water from a municipal water supply thought to be contaminated by feces of infected cats. To protect the U.S. water supply, the U.S. Environmental Protection Agency (EPA) is developing methods to detect Toxoplasma oocysts in municipal water. ARS has established a CRADA with EPA to develop methods for detecting T. gondii in water. The ARS obligation is to supply Toxoplasma oocysts and antibodies for the development of the detection methods.

2. How serious is the problem? Why does it matter?

Approximately 3000 children are born congenitally infected with T. gondii every year in the U.S. and the cost of raising these children is more than 5.6 billion dollars annually.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research supports ARS programs in Animal Health (103) and Food Safety (108), by developing methods to detect and prevent exposure of

animals and humans to parasitic diseases.

4. What was your most significant accomplishment this past year?

The CRADA was initiated September 1, 1998 and as yet there are no accomplishments.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402335 Year: 98 Project Number: 1265-32000-045-10 R
Mode Code: 1265-40-00 STP Codes: 3.2.1.3 100%
NATL PROG(S) 103 Animal Health 40%
108 Food Safety, (animal products) 60%

The CRADA was initiated September 1, 1998 and as yet there are no accomplishments.

6. What do you expect to accomplish during the next year?

EPA will do the developmental work. The ARS obligation is to supply oocysts.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149677 Year: 98 Project Number: 1265-32000-047-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.2 30% 3.2.1.4 70%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: INTEGRATED CONTROL OF AVIAN COCCIDIOSIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149677 Year: 98 Project Number: 1265-32000-047-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.2 30% 3.2.1.4 70%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production Systems National Program (102) through the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

This project integrates several research components and there has been more than one significant accomplishment achieved during the past year. In natural product studies, dietary supplementation with 110 to 200 ppm curcumin was found to have beneficial effects against challenge infection with two small-intestinal species of coccidia (E. acervulina and E. maxima). This adds another dietary supplement to those already identified to reduce the impact of coccidia infection. We have previously developed a customized "live vaccine" for avian coccidiosis. Extending on this work, field trial studies done with Perdue Inc., Salisbury, MD, showed that use of a combination of immunization of birds with anti-coccidial resistant coccidial strains coupled with anticoccidial medication gives the greatest increase in broiler flock performance during the late spring to early fall time period. It was also found that flocks on this type of combination treatment would outperform other flocks treated with anticoccidial medication alone throughout an entire year of growout.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

During the 3 year life of this project we have several short and long term accomplishments directed toward measures for the control of avian coccidiosis. Accomplishments classified under short term control include demonstrating that the addition of betaine, a sugar beet by-product, to the anticoccidial salinomycin gives increased efficacy against coccidial infection. We found that n-3 fatty acid dietary-induced oxidative stress and use of the antioxidants gamma-tocopherol and curcumin reduces lesions of two different species of coccidia, so that dietary control of coccidiosis is feasible. We showed that a new premix formulation of the

Accession: 0149677 Year: 98 Project Number: 1265-32000-047-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.2 30% 3.2.1.4 70%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

anticoccidial compound Bio-Cox was more effective in the control of coccidia than previous formulations. These studies have been used to introduce this improved premix to the poultry industry for coccidiosis control. We determined that immunization with anticoccidial resistant strains of coccidia at the hatchery would improve bird performance in commercial broiler growout houses, when used in combination with various anticoccidial compounds. We also used a gamma-irradiated live oocyst vaccine delivered by a gel-immunization technique to achieve increased bird performance in field trial studies. These latter three findings have been implemented by the poultry industry as an effective means of coccidial control and have been used to vaccinate over 70 million birds. Major accomplishments in long term research include the finding that pretreatment of cell lines in vitro with a monoclonal antibody directed against refractile bodies of Eimeria sporozoites significantly inhibited invasion by two species of coccidia. This research will aid in identifying attachment sites for parasite penetration and further our knowledge of host-parasitic interactions. We developed biochemical and immunohistochemical detection assays for nitric oxide synthase, a known indicator of the inflammatory response. This research will help to determine the effects of non-specific and specific directed inflammatory responses on both the infected chicken and on coccidial development, key information for vaccine development. Finding that the interaction of multiple species of coccidial parasites will effect the degree of sporozoite invasion in birds also effects the way in which control strategies will be developed. As the next generation of vaccines is developed we have found that use of a DNA plasmid injection technique is an effective delivery system for recombinant coccidial antigen immunization in birds.

6. What do you expect to accomplish during the next year?

We plan to establish the relationships between refractile body antigens and invasion using coccidial genera having refractile bodies, crystalline bodies or lacking refractile bodies. This will help to determine the function of these organs and their suitability as a target

for control. We will determine if use of spray cabinet exposure to coccidial oocysts will elicit a sufficiently protective immune response to broiler birds in field trial studies to warrant its use for vaccination. We will prepare live oocyst vaccine using anticoccidial resistant strains of coccidia to begin full scale immunization of 4-5 million broiler birds/month.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Accession: 0149677 Year: 98 Project Number: 1265-32000-047-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.2 30% 3.2.1.4 70%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Three different types of live oocyst vaccination systems have been directly transferred to the poultry industry. Use of reformulated live oocyst vaccines containing immuno-variant strains of the same species of coccidia and a combination of drug resistant coccidial strain vaccination with anticoccidial medication is currently being used by poultry producers throughout the U.S. to vaccinate approximately 1.5 million birds/month. Gamma-irradiated oocyst vaccination is scheduled to begin field trial testing by spring of 1999. These types of immunization systems will become more integrated into poultry production as the anticoccidial compounds decrease in efficacy. The addition of betaine to feed with the use of the anticoccidial salinomycin is now an accepted practice in the poultry industry.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

"A role of n-3 fatty acids form fish oil in the control of cecal coccidiosis" 39th Annual Fisheries Sym., Baltimore, MD, March, 1998
"Dietary modulation of avian coccidiosis. "Hoffman-La Roche Inc., Nutley NJ, June 10, 1998
"Use of Ionogrow vaccination in the control of avian coccidiosis. Elanco Anim. Health & Perdue Inc. Eli Lilly, Indianapolis, IN, June 7-8, 1998"
"Coccidiosis in Turkeys." In: Proc. 4th Internal. pp. 9-14. Roche Turkey Enteric Health Symposium, Oct. 26-30, 1998, Short Hills, NJ

PUBLICATIONS:

01.
ALLEN, P.C. 1998. Effects of in vivo treatment of coccidia-infected chickens with methylisothiourea. Exp. Bio. 98. FASEB J 12:A573.

02.
ALLEN, P.C. 1998. Segmented filamentous bacteria (SFB): friends or foes? A brief review. 36 Ann. Mt. Assoc. Gnotob. P. 14.

03.
ALLEN, P.C. and LILLEHOJ, H.S. 1998. Genetic influence on nitric oxide production during Eimeria tenella infections in chickens. Avian Dis. 42:397-403.

04.
ALLEN, P.C. and DANFORTH, H.D. 1998. Effects of dietary supplementation with n-3 fatty acid ethyl esters on coccidiosis in chickens. Poultry Sci. 77:1631-1635.

Publications: (Continued)

05.
ALLEN, P.C., DANFORTH, H.D. and AUGUSTINE, P.C. 1998. Dietary modulation of avian coccidiosis. Int. J. Parasitol. 28:1131-1140.

06.
AUGUSTINE, P.C. 1998. Avian Eimeria: Invasion in cells pretreated with an antisporozoite monoclonal antibody. Prog. and Absts. 73rd Ann. Mt. Amer. Soc. Parasitol. p. 87.

07.
AUGUSTINE, P.C., CLINE, P.N. and DANFORTH, H.D. 1998. Use of parasite-specific monoclonal antibodies to study invasion ... Eimeria gruis in the Florida Crane (Grus canadensis). J. Zool. Wild. Med. 29(1):21-24.

08.
AUGUSTINE, P.C. and JENKINS, M.C. 1998. Effect of conditioned media from chicken and turkey intestinal cell cultures on invasion by three species of avian coccidia. J. Euk. Microbiol. 45:344-346.

09.
DANFORTH, H.D. 1998. Use of live oocyst-based vaccines in the control of avian coccidiosis: Experimental studies and field trials. Int. J. Parasitol. 28:1099-1109.

10.
DANFORTH, H.D. and LEE, E-H. 1998. Use of live oocyst-based vaccines in avian coccidial control. Chinese J. Vet. Parasit. 6:25-27.

11.
MARTIN, A.G., DANFORTH, H.D., JAYNES, J.M. and THORTON, J. 1998. Evaluation of the effect of peptidyl membrane interactive molecules (Peptidyl-MIMs) on avian coccidia. Parasit. Res. 84:550-556.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0148746 Year: 98 Project Number: 1265-32000-047-06 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.2 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: EFFECT OF BETAINE ON INVASION AND DEVELOPMENT OF AVIAN COCCIDIA IN CELL CULTURE AND CHICKENS, ...

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Coccidiosis, one of the major enteric diseases of chickens, turkeys, and game birds, costs the poultry industry hundreds of millions of dollars each year despite currently used control measures. New methods of control, to be used in place of, or as adjuncts to, chemotherapy are needed. Natural products, that are renewable commodities, are especially attractive as feed additives that offer potential for control. This project focuses on the use of betaine, a product of the sugar beet, in conjunction with anticoccidial drugs, as a defense against coccidiosis.

2. How serious is the problem? Why does it matter?

The expense of developing and clearing new drugs, the growing drug-resistance of the coccidia, and the demand by consumers for food animals grown free of drugs, strongly suggest that alternative control measures need be developed. The coccidia are cosmopolitan in distribution, with the range of each species limited only by the range of its natural host. Without adequate control, coccidiosis could decimate the poultry industry worldwide.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research meets the objectives of National Programs in Animal Health (103) and Animal Production Systems (102) by developing new methods for the control of poultry diseases and enhancing the productivity

(performance) of birds.

4. What was your most significant accomplishment this past year?

The discovery that dietary betaine enhanced nutrient absorption in coccidia-infected chickens was important. Even more significant was the finding that the magnitude of the effect differed with the anticoccidial sensitivity profile of the coccidia because isolates of coccidia from commercial operations display a continuum of drug sensitivities.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

A series of studies showed that betaine, in combination with salinomycin, protected chickens against the negative impact of coccidiosis on performance parameters (weight, feed conversion, etc.). The enhancement in performance was due, in part, to a direct effect of betaine on the invasion and development of the coccidia. The major impact of the compound, however, was directed at the chicken. Intestinal cells remained hydrated in infected birds, supporting normal cell structure and function in the face of coccidial infection. This information has ramifications far beyond the alleviation of the clinical signs of coccidiosis; the negative impact of any enteric disease that disturbs the osmotic balance and function of intestinal cells could potentially be decreased by including betaine in the diet.

6. What do you expect to accomplish during the next year?

Natural products shown to have anticoccidial effects often exert these effects against a single species. Plans for next year will be 1) to identify synergistic or additive effects between betaine and other natural products shown to have protective effects in coccidia-infected chickens, and 2) to formulate cocktails of products that will be active against several species of coccidia.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Partly as a result of this work, betaine is being widely used in the U.S. and elsewhere as a feed additive for chickens. Strains of parasites have been sent to scientists in Kantvik and Helsinki, Finland which are being used in parallel studies with betaine. As more is known about the product and its effects on animals and animal pathogens, its use is likely to increase. Betaine is a natural product, derived from sugar beets, so there are few constraints on its use or on the expected supply of the compound.

8. List your most important publications and presentations, and articles

written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Effect of Betaine in Coccidia-infected Chickens Betaine and Coccidiosis Betafin Research Seminar, Atlanta, GA, January 17-18, 1998.

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0148353 Year: 98 Project Number: 1265-32000-047-07 R
Mode Code: 1265-40-00 STP Codes: 3.2.1.2 50% 3.2.1.3 50%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: METHODS FOR THE CONTROL OF COCCIDIOSIS IN GAMEBIRDS

Period Covered From: 01/98 To: 08/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Production of game birds (pheasant, quail, etc.) is a growing industry in the U.S. and elsewhere. These birds are extremely susceptible to coccidial infection, but there are few if any drugs approved by the FDA for use against coccidiosis in game birds. This project was designed to provide information for the approval of 2 drugs, Clopidol and Lasalocid, for use in game birds.

2. How serious is the problem? Why does it matter?

Levels of drugs used for one breed of bird may be toxic or leave residues in other breeds of birds. Therefore, information on efficacy and safety garnered for domestic poultry is not directly applicable to game birds.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research meets the objectives of National Programs in Animal Health (103) and Animal Production Systems (102) by developing new methods for the control of poultry diseases and enhancing the productivity (performance) of birds.

4. What was your most significant accomplishment this past year?

Tissues were collected from pheasants medicated with Clopidol and Lasalocid for the measurement of drug residues. Because of the lack of

an approved standard, the tissues had not been assayed when the agreement was terminated.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Efficacy and safety levels of 2 anticoccidial drugs, Clopidol and Lasalocid, were determined in pheasants. Tissues were collected from pheasants medicated with the appropriate levels of each drug but have not been assayed. This information should contribute to the approval of

Accession: 0148353 Year: 98 Project Number: 1265-32000-047-07 R
Mode Code: 1265-40-00 STP Codes: 3.2.1.2 50% 3.2.1.3 50%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

the drugs for use in pheasants.

6. What do you expect to accomplish during the next year?

Agreement terminated.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Drugs that protect against coccidiosis have been used successfully for domestic poultry and it is expected that they will be used successfully by the game bird industry when they are approved by the FDA.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400172 Year: 98 Project Number: 1265-32000-047-09 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: COCCIDIOSIS CONTROL IN CHICKENS

Period Covered From: 01/98 To: 11/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400172 Year: 98 Project Number: 1265-32000-047-09 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

Genetic fragments coding for luciferase expression or recombinant coccidial antigens were inserted into DNA plasmids and injected via jet gun into skeletal muscle of broiler birds to study the potential for eliciting a protective immune response against coccidial infection. Battery cage studies have shown that luciferase is expressed in the skeletal muscle of birds and that partial protection to a challenge coccidial infection does occur in birds injected with the DNA plasmids containing the coccidial inserts.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Avian coccidial oocysts have been harvested monthly in large numbers (> 6 X 108) to be used in biochemical and antigenic studies on the control of avian coccidiosis. Preliminary results with plasmid DNA injection showed that luciferase was expressed in significant amounts up to 15 days after plasmid injection, which demonstrated that this delivery system could be utilized in chickens. In addition, injection of plasmids containing recombinant coccidial antigen demonstrated that this type of

immunization system could be used to immunize birds against coccidial infection.

6. What do you expect to accomplish during the next year?

This project was terminated on 1 November, 1998 but has been replaced by a new project (No. 1265-32000-047-12 T), which has continued the work on biochemical and antigenic studies on avian coccidia. As will be discussed in a related project report, further battery trial studies on the use of plasmid DNA injection vaccination have shown that titration

Accession: 0400172 Year: 98 Project Number: 1265-32000-047-09 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

of number of plasmids given is essential for producing a protective immune response to coccidial infection.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

To date, the use of DNA plasmid injection technology for immunization against avian coccidiosis has not been transferred to the poultry industry because the research has not progressed beyond evaluation in caged battery trials. If results continue to be positive, it is conceivable that floorpen tests using this type of immunization could occur within 2 years. Positive results with floorpen trials could then lead to field testing of plasmid DNA immunization. Major constraints in developing DNA plasmid immunization for avian coccidia include lack of knowledge on the amount and kind of antigen needed to elicit significant protection against coccidial infection, and lack of knowledge as to whether birds will develop a protective immune response when grown under conditions found in broiler growout houses used by the poultry industry.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

"Control of Avian Coccidiosis." Arkansas Farm Bureau, March 16, 1998

PUBLICATIONS:
01.
DANFORTH, H.D., JENKINS, M.C. and AUGUSTINE, P.C. 1998. Evaluation of luciferase expression in skeletal muscle of chickens injected with plasmid DNA. Prog. and Absts. 73rd Ann. Mt. Amer. Soc. Parasitol. p. 96.

Approved: D.F. COLE Date: 02/99

Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400494 Year: 98 Project Number: 1265-32000-047-10 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: ANTICOCCIDIAL CONTROL OF AVIAN COCCIDIOSIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups is increasing to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400494 Year: 98 Project Number: 1265-32000-047-10 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

Battery and floorpen trials using male broiler chickens have shown that a new premix formulation of the ionophorous anticoccidial Salinomycin (Bio-CoxTM) was effective against a number of coccidial strains collected from different geographical areas of the U.S. It was also found that the addition of Roxarsone (3-NitroTM) to another ionophorous anticoccidial Lasalocid (AvatecTM) caused a decrease in bird performance in battery trials after challenge with certain field isolates of coccidia, compared to birds challenged with the same isolates of coccidia and medicated with Lasalocid only.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

The evaluation of the new premix formulation of Bio-Cox has shown that this anticoccidial is more uniformly mixed in the feed which actually increases the efficacy of Salinomycin against coccidial field strains. This premix has now been cleared for use in the poultry industry and is being used for effective control of coccidia in bird flocks. Use of Roxarsone in combination with Lasalocid is still being evaluated in

floorpen trials to determine if a drop in bird performance will occur similar to that seen in battery trials. A positive correlation with the floorpen trials would mean that this combination of medications should not be used in certain poultry growout areas of the U.S. These studies have and will give a greater understanding of the interaction of combinations of medications with coccidial control.

6. What do you expect to accomplish during the next year?

We will determine, in floorpen trial studies, if the use of Roxarsone in

Accession: 0400494 Year: 98 Project Number: 1265-32000-047-10 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

combination with Lasalocid will cause a decrease in bird performance when challenged with specific field strains of coccidia obtained from different geographical areas of the U.S.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The new premix formulation of Bio-Cox is now being used by the poultry industry for anticoccidial control. Constraints on the use of this anticoccidial hinge mainly on the development of resistance of the parasites. However, with the use of novel shuttle systems in which anticoccidial treatments are rotated approximately every 4 to 6 months, the efficacy of Salinomycin may be extended for a number of years.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:
01.
PECELUNAS, K., DANFORTH, H.D., SCHILDKNECHT, E. and DAVIS, S. 1998. Efficacy evaluation of Lasalocid and Roxarsone ... geographical field strain isolates of Eimeria acervulina. Poultry Sci. 77(1):58.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400395 Year: 98 Project Number: 1265-32000-047-11 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.5 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: IMMUNOLOGICAL VARIABILITY OF AVIAN COCCIDIA

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400395 Year: 98 Project Number: 1265-32000-047-11 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.5 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

Battery trials using male broiler chickens have shown that there is a variability in immunological cross-protection between 5 geographically different strains of Eimeria maxima, a mid-intestinal species of avian coccidia. Three of these strains were found to be cross-protective, but the other two strains showed no cross-protection between themselves or among the other three strains. Analysis of the infra-specific variation among the sporozoite stage of these 5 strains of coccidia showed that their phylogenetic relationships did not correlate with the immunological cross-reactivities of these strains. This work is integral in designing custom live vaccines for the poultry industry.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Variability of immunological cross-protective potential between 5 different strains of Eimeria maxima demonstrated that it may not be possible to produce a uniform live oocyst vaccine for use in the poultry industry. Vaccines may have to be formulated for regional use in order to be elicit a protective immune response in bird flocks. In addition,

when the sporozoite stage of these strains was examined for phenotypic and genotypic variation using protein profiles, random amplified polymorphic DNA-PCR analysis and internal transcribed spacer regions of the rRNA genes, no correlation was seen between the immunological cross-reactivities and the phenotypic and genotypic variation. These findings indicate that other developmental stages of the life cycle of these parasites must be examined to determine the extent of genetic variation.

6. What do you expect to accomplish during the next year?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400395 Year: 98 Project Number: 1265-32000-047-11 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.5 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

We will determine the dynamics and extent of lymphocyte infiltration in birds challenged with homologous and heterologous strains of E. maxima and begin analysis of the infraspecific variation of the schizont and sexual stages of different strains of E. maxima.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Knowledge that there is variation in cross-protective potential among different geographical isolates of the same species of coccidia has been directly transferred to both the vaccine and poultry industries of the U.S. This has resulted in the reformulation of live oocyst vaccines for use in poultry production facilities in Northern Florida, Central Arkansas and the DelMarVa peninsula. To date, over 50 million birds have been vaccinated with the reformulated vaccines.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

"Internal and External Factors Impacting Coccidiosis." First International Seminar on Coccidiosis sponsored by the Peruvian Veterinarian Association in Lima, Peru, June 18-19, 1998

PUBLICATIONS:
01.
BARTA, J.R., COLES, B.A., SCHITO, M.L., FERNANDO, M.A., MARTIN, A. and DANFORTH, H.D. 1998. Analysis of infraspecific variation among ... of Eimeria maxima from North America. Inter. J. Parasitol. 28:485-492.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400922 Year: 98 Project Number: 1265-32000-047-12 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: OOCYSTS PRODUCTION OF EIMERIA TENELLA

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400922 Year: 98 Project Number: 1265-32000-047-12 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

Over 72 billion Eimeria oocysts have been produced and sent to our collaborator to continue basic studies on the biochemical and antigenic makeup of the parasite. DNA plasmids containing genetic fragments coding for coccidial antigens were injected via gene gun into skeletal muscle of broiler birds to study the potential for eliciting a protective immune response against coccidial infection. Battery cage studies have shown that partial protection to a challenge coccidial infection does occur in birds injected with plasmids containing the coccidial inserts for three separate antigens, but that injection of larger amounts of plasmids (1 to 10ug/bird) will not effectively immunize birds against coccidial infection.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Avian coccidial oocysts continue to be harvested monthly in large numbers (> 6 X 108) for use in biochemical and antigenic studies on the potential control of avian coccidiosis. Replication experiments on plasmid DNA injection vaccination have shown that this type of

immunization technique could be used to immunize birds against coccidial infection, but that larger amounts of DNA plasmid injection actually exacerbate the infection or produce a negative effect on the birds after challenge infection. This latter observation has led to speculation that increased antigen presentation of the 3 coccidial antigens (designated SZ240, MZ250 and SO7) through injection of larger amounts of plasmid DNA (1 to 10ug plasmid DNA/bird) would in effect drive the bird's immune system to negatively impact weight gain and feed conversion. These results indicate that careful titration of the amount of DNA plasmid injected is necessary to achieve the desired protection against the

Accession: 0400922 Year: 98 Project Number: 1265-32000-047-12 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

parasite challenge.

6. What do you expect to accomplish during the next year?

This project replaces by an older project (No. 1265-32000-047-9 T), which initiated the work on biochemical and antigenic studies on avian coccidia. Further battery titration trial studies on the use of plasmid DNA injection/vaccination will be attempted to determine the correct amount of plasmid that must be given to elicit a protective immune response to coccidial infection.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The use of DNA plasmid injection technology for immunization against avian coccidiosis has not been transferred to the poultry industry because the research has not progressed beyond evaluation in caged battery trials. If results from the titration experiments are positive, it is conceivable that floorpen testing of this type of immunization could occur within 2 years. Positive results with the floorpen trials could then lead to field trial testing of plasmid DNA immunization. Major constraints in developing DNA plasmid immunization for avian coccidia include the continued lack of knowledge on the amount and kind of antigen needed to elicit significant protection against coccidial infection, and how to modulate the immune response to insure that broiler birds will elicit a protective and not a potentially destructive immune response against the parasites.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

"Immunity Response: Infection by Coccidiosis." First International

Seminar on Coccidiosis, sponsored by the Peruvian Veterinarians Association in Lima, Peru, June 18-19, 1998

PUBLICATIONS:

01.

JENKINS, M.C., ALLEN, P.C., DANFORTH, H.D. and AUGUSTINE, P.C. 1998. Immunization of chickens ... antigen via jet-gun ... immunity against coccidiosis. Proc. 43rd Annual Mtg., Am. Assoc. Vet. Parasitol. p. 47.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401068 Year: 98 Project Number: 1265-32000-047-13 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.5 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: MEASUREMENT OF GUT-IMMUNOLOGICAL/PHYSIOLOGICAL RESPONSE TO AVIAN COCCIDIOSIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly economic negative impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce losses caused by avian coccidia.

Accession: 0401068 Year: 98 Project Number: 1265-32000-047-13 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.5 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

Using-type chambers were utilized to measure coccidial antigen induced chloride ion (Cl-) secretion in intestinal tissue segments from nonimmunized and coccidial-immunized birds. Measurable increases in Cl- secretory activity were seen in ileal segments from immunized birds exposed to soluble coccidial antigen. Minimal increases in secretory activity was seen in ileal segments from nonimmunized control birds. These results suggest the ion secretion by intestinal mucosal epithelium in response to antigen challenge in vitro may serve as an indicator of immunity to coccidia in chickens.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Using an electrophysiological measurement of intestinal anaphylaxis (antigen-induced Cl-secretion in Using chambers), a type I hypersensitivity reaction was seen in chicken intestine from birds immunized with BSA (bovine serum albumin), following serosal challenge. These results indicate that this type of model could be used to measure, at the mucosal and serosal level, the immune response of immunized birds

to coccidial antigen. Subsequent studies using intestinal segments from immunized birds have shown an increase in the Cl-secretory activity of the mucosal epithelium after stimulation with soluble coccidial antigen. These positive results now make it possible to study the variability in immune response in specific areas of the intestine not only between immunovariant strains of the same species of coccidia but also between different species of the parasite.

6. What do you expect to accomplish during the next year?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401068 Year: 98 Project Number: 1265-32000-047-13 S
Mode Code: 1265-40-00 STP Codes: 3.2.1.5 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

We will measure the Cl-secretory activity of upper, middle and lower intestinal segments from birds immunized with different species of coccidia after exposure to soluble antigen from the same or different species to determine the extent and specificity of intestinal mucosal response.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

This specific cooperative agreement has been in place for approximately one year and the progress to date has shown that this model has great potential for understanding the intestinal immune response to coccidial infection. No technology has been transferred to the poultry industry, but, as the research continues, the information obtained can be directly applied to research efforts to produce more effective immunological controls for avian coccidia.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:
01.
CALDWELL, D.J., MCELROY, A.P., REINAP, R.A. and DANFORTH, H.D. 1998. Gut immunity in the chicken: Responsiveness of the mucosal epithelium to antigenic stimulation. Poultry Sci. 77(1):41.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401701 Year: 98 Project Number: 1265-32000-047-14 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

Title: EIMERIA OOCYST PRODUCTION FOR COCCIDIAL CONTROL

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401701 Year: 98 Project Number: 1265-32000-047-14 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

Over 24 billion oocysts of two different strains of Eimeria maxima have been produced and sent to our collaborator to continue basic studies on the development of a delivery system for live oocyst vaccination of chickens.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Avian coccidial oocysts have been harvested monthly in large numbers (greater than 1 billion) for use in developing a new and novel delivery system (using yolk sac diverticulum injection) for live oocyst vaccination of commercial chicken flocks.

6. What do you expect to accomplish during the next year?

We will continue to produce and supply (through May, 1999), one billion oocysts of two different strains of E. maxima for use in live oocyst vaccination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

No transfer of technology to the poultry industry has occurred, but significant progress has been made so that a final decision will be made by the fall of 1999 whether to begin field trial tests on the yolk sac injection delivery method for live oocyst immunization of broiler birds. If field trials give positive results, this technology could be

Accession: 0401701 Year: 98 Project Number: 1265-32000-047-14 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

available to the poultry industry within 3 years.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401934 Year: 98 Project Number: 1265-32000-047-16 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

Title: EIMERIA TENELLA OOCYSTS PRODUCTION

Period Covered From: 08/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis, an intestinal disease caused by infection with intracellular protozoan parasites of the genus Eimeria, is estimated to cost the U.S. poultry industry over $450 million annually. This disease is partially controlled in commercially grown chickens and turkeys by use of anticoccidial compounds in the feed. However, the coccidia are steadily becoming more resistant to all anticoccidials now used in the poultry industry. Except for two anticoccidials that are in the final stages of FDA clearance, no new anticoccidial compounds are being developed. New directions in the control of these parasites is urgently needed. Four areas of research have been targeted for improved coccidial control. Research in applied methods, which will provide the industry with technology to control coccidiosis in the short-term, includes evaluation and creative utilization of both new and old classes of anticoccidials, dietary factors, natural products and live oocyst vaccines. Long-term research is conducted in three other areas including understanding host factors (including physiological and inflammatory responses) which contribute to natural resistance to coccidiosis, developing novel targets for control by understanding the processes of cellular invasion (attachment and internalization) and development, and use of recombinant DNA technology to produce effective vaccines.

2. How serious is the problem? Why does it matter?

Most coccidia are resistant to existing drugs, and no new drugs are in the FDA clearance process. Due to changes in the animal health/pharmaceutical industry, few companies are even considering drug

discovery for the coccidia. Even though current control measures for coccidia in the poultry industry are not effective, no new anti-coccidials will become available in the foreseeable future. In addition to a lack of suitable drugs, pressure is increasing within consumer and legislative groups to eliminate the use of most drugs, including the ionophorous anticoccidial compounds, in agricultural animals. This critical dilemma predicts an increasingly negative economic impact on the poultry industry caused by the coccidia, and requires the development of both short and long term control strategies to effectively reduce these losses.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401934 Year: 98 Project Number: 1265-32000-047-16 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research addresses prevention of disease in poultry through the use of anti-coccidial drugs, vaccines, and natural products which are objectives of the Animal Disease National Program (103). The research addresses the program objective of reducing losses due to animal parasites using cost-effective and integrated systems. Research also supports the Animal Production systems National Program (102) though the use of feed additives and other methods of coccidia control, to maximize bird performance. Management decision aids for coccidia control are being developed to benefit producers.

4. What was your most significant accomplishment this past year?

This is a new project that was set up 1 August, 1998 to replace project No. 1265-32000-047-12T. To date, only one battery trial on immunization of chickens with DNA plasmids containing genetic fragments coding for coccidial antigens injected via jet gun into skeletal muscle has been conducted. This study again showed that injection of larger amounts of DNA plasmid (1 to 10ug/bird) will not immunize birds against coccidial infection.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Replication experiments on titration of amount of plasmid DNA needed for vaccination against coccidial infection will be continued to achieve the desired protection against the parasite challenge.

6. What do you expect to accomplish during the next year?

We will conduct additional battery and floorpen titration studies on the use of plasmid DNA injection vaccination to determine the correct amount

of plasmid and type of antigen that must be given to elicit a protective immune response to coccidial infection.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The use of DNA plasmid injection technology for immunization against avian coccidiosis has not been transferred to the poultry industry

Accession: 0401934 Year: 98 Project Number: 1265-32000-047-16 T
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 102 Animal Production Systems 30%
103 Animal Health 70%

because the research has not progressed beyond evaluation in caged battery trials. If results from the titration experiments are positive, floorpen evaluation of this type of immunization will then be undertaken. Positive results with the floorpen trials could then lead to field trial testing of plasmid DNA immunization in the poultry industry. Major constraints in developing DNA plasmid immunization for avian coccidia include the continued lack of knowledge on the amount and kind of antigen need to develop significant protection against coccidial infection, modulation of the immune response to ensure that broiler birds will elicit a protective and not a potentially destructive immune response against the parasites and how interaction of this type of vaccination with exposure of the bird to other diseases of poultry will effect the degree of protection against coccidial infection. Question 8: "Control of Avian Coccidiosis." Maryland Secretary of Agriculture Delegation, July 30, 1998.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

"Control of Avian Coccidiosis." Maryland Secretary of Agriculture Delegation, July 30, 1998.

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149709 Year: 98 Project Number: 1265-32000-052-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 70% 3.2.2.7 30%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

Title: ECOLOGICALLY-BASED TECHNOLOGIES FOR CONTROLLING IXODES SCAPULARIS AND REDUCING LYME DISEASE

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The incidence of Lyme disease and human granulocytic ehrlichiosis has been increasing in the United States. This increase has been attributed to the expansion of populations of white-tailed deer and a concomitant increase in numbers of the deer (black-legged) tick, vector of these diseases. Area-wide applications of insecticides in tick habitats are often impractical and environmentally undesirable. As an alternative, populations of Ixodes scapularis can be reduced by targeting applications of acaricides or biological agents to deer hosts, or to areas of high tick infestation. We are using topical application device technology to assess the feasibility of treating deer with chemicals which kill ticks as a way to reduce Lyme tick populations. At the same time, we are identifying and testing biological agents such as nematodes and entomopathogenic fungi for effects on Ixodes scapularis. These biological agents could be used in topical application devices, in lieu of pesticide, or might be sprayed in residential areas. We are also determining the feasibility of disrupting the tick deer association by manipulating behavior-mediating chemicals.

2. How serious is the problem? Why does it matter?

Lyme disease is a debilitating illness that afflicts thousands of Americans annually. Without timely and appropriate use of antibiotics, Lyme disease can develop into a serious chronic condition that can result in considerable loss of work and school hours. Deer ticks have expanded their range and increased in numbers during the past 20 years, becoming entrenched in suburban areas where white-tailed are

increasingly abundant. By controlling tick populations or disrupting their capacity to find hosts or mates, tick bites and Lyme disease can be reduced.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The goals of this research are to discover and develop new environmentally acceptable technologies that reduce the risk of Lyme disease and other tick borne illnesses in humans and domesticated

Accession: 0149709 Year: 98 Project Number: 1265-32000-052-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 70% 3.2.2.7 30%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

animals. This work supports the objectives and is a component of National Program 104, Arthropod Pests of Animals and Humans.

4. What was your most significant accomplishment this past year?

We demonstrated the effectiveness of the entomopathogenic fungus, M. anisopliae, under field conditions, against I. scapularis. We found that two applications of M. anisopliae (at about 340,000 spores per square cm) reduced free-living nymph populations in experimental plots by 50%. Three applications also reduced naturally occurring populations of larvae.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

A device, the tick sweep, was developed for sampling host-seeking nymphal I. scapularis in dense vegetation, where it captures more ticks than traditional methods. The device captures ticks in advance of the operator and does not require stooping or kneeling in tick infested habitats. Several of the devices were donated to researchers at U.S. Army Walter Reed Medical Center at their request. By releasing marked host-seeking I. scapularis nymphs and adults in large host-exclusion cages we showed that these ticks, especially the adults, were capable of dispersing distances of several meters by their own locomotion. We found that silt fencing prevented dispersal of host-seeking nymphs and adults of I. scapularis and could reduce the risk of tick bite in lawns and pastures. We described the patterns of attachment of adult I. scapularis to the body regions of deer and horses. This information supported the development of host self-treatment devices for controlling deer ticks. An estimated 90% or more of adult I. scapularis feed on white-tailed deer, and we found that 90% of the adult I.scapularis attach on areas of deer most likely to come in contact with the insecticide-impregnated rollers on the self-treatment device. In field and laboratory studies, substances associated with the external glands of white-tailed deer were found to influence where host-seeking I. scapularis adults wait for

hosts. These sort of substances (kairomones) were also found to influence the behavior of other species of ticks. IPM Technologies, Inc. Seattle, WA is initiating further studies of the chemistry on the deer gland substances. Certain fungi and nematodes pathogenic to arthropods are being investigated for activity against I. scapularis. Laboratory evaluations of nematodes belonging to the genus Steinernema and of Metarhizium fungi have given positive results and indicate a need for further field studies of these pathogens. Under a material transfer agreement with Ecoscience, Inc. East Brunswick, NJ, M. anisopliae, a known entomopathogen licensed for use against termites, was field tested

Accession: 0149709 Year: 98 Project Number: 1265-32000-052-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 70% 3.2.2.7 30%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

against I. scapularis larvae. The efficacy of the "4-poster" deer self-treatment device is being tested in large scale field trials for area-wide control of I. scapularis and the lone star tick, Amblyomma americanum, vector of monocytic ehrlichiosis.

6. What do you expect to accomplish during the next year?

We plan to continue the evaluation of deer self-treatment devices for area wide control of I. scapularis at three sites in Maryland, and will have our first indication of their effectiveness in lowering tick populations (larval numbers). Field testing of nematodes and fungi against I. scapularis will be conducted to determine the feasibility of using these biological control agents in conditions of suburbia where Lyme disease is endemic. We are presently evaluating the effects of M. anisopliae on 2,000 fed I. scapularis larvae confined in a natural leaf litter microhabitat. Morbidity, mortality and development (molting) of the treated ticks and an equal number of untreated ticks and will be checked monthly through June, 1999. A similar trial using nymphs is scheduled to start in April, 1999, and a replicate trial against free-living larvae and nymphs will take place in April through October, 1999. In a collaborative effort with the Insect Chemical Ecology Laboratory, the kairomonally active component(s) of deer or canine coat substances will be isolated so that they can be field tested and synthesized if necessary.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

We surveyed the Sherwood Forest community in Maryland for I. scapularis. We established deer self-treatment device study sites in two communities, Gibson Island and Phoenix, MD. In the latter locality the devices are located on the premises of cooperating homeowners. We distributed literature to Phoenix residents, discussed the project with

residents and presented talks to the Gibson Island Association. The area wide tick control project and biological control studies were featured in articles in the Baltimore Sun. IPM Technologies, Inc., Seattle, WA expressed an interest in cooperative work on kairomones associated with deer leg glands. The preparation of M. anisopliae, used in the field trials reported here, is commercially available as "Bioblast" and is supplied to us by Ecoscience. Any application method devised by us for this fungal preparation that controls populations of I. scapularis will be transferred to Ecoscience. It is envisioned that this biocontrol technology will be best suited for relatively small areas such as wooded

Accession: 0149709 Year: 98 Project Number: 1265-32000-052-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 70% 3.2.2.7 30%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

residential lots typical of suburban settings with high incidences of Lyme disease.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Agricultural Research, 1998, Tackling ticks that spread Lyme disease. 46: 22-24.
Baltimore Sun, Jan. 19, 1998, Patuxent Institution inmates product will help fight the spread of Lyme disease.
Baltimore Sun, June 9, 1998, Worms fungi lead Lyme Disease fight.

PUBLICATIONS:

01.
CARROLL, J.F., MILLS, G.D. and SCHMIDTMANN, E.T. 1998. Patterns of activity in adult host-seeking Ixodes scapularis (Acari: Ixodidae) and host-produced kairomones. J. Med. Entomol. 35:11-15.

02.
SCHMIDTMANN, E.T., CARROLL, J.F. and WATSON, D.W. 1998. Attachment site patterns of Ixodes scapularis (Acari: Ixodidae) on white-tailed deer and horses. J. Med. Entomol. 35:59-63.

03.
CARROLL, J.F. 1998. Kairomonal activity of white-tailed deer metatarsal gland substances: a more sensitive bioassay using Ixodes scapularis (Acari: Ixodidae). J. Med. Entomol. 35:90-93.

04.
SCHMIDTMANN, E.T., SCHLATER, J.L., MAUPIN, G.O. and MERTINS, J.W. 1998. Vegetational ... host-seeking adult blacklegged ticks (Acari: Ixodidae), on dairy farms in northwestern Wisconsin. J. Dairy Sci. 81:718-821.

05.

MARTIN, P.A.W. and SCHMIDTMANN, E.T. 1998. Isolation of microbes from Ixodes scapularis (Acari: Ixodidae), the vector of Lyme disease in the eastern United States. J. Econ. Entomol. 91:864-868.

06.
HILL, D.E. 1998. Entomopathogenic nematodes as control agents of developmental stages of the black-legged tick, Ixodes scapularis. J. Parasitol. 84:1124-1127.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400643 Year: 98 Project Number: 1265-32000-052-02 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 100%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

Title: CONTROL METHODS FOR THE DEER TICK VECTOR OF LYME DISEASE BASED ON TICK-DEER HOST INTERACTIONS

Period Covered From: 01/98 To: 09/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The major issue this project addresses is the threat of disease to humans and domestic animals caused by the deer tick. This project is intended to resolve this issue by identifying ecological factors that limit population growth in deer ticks which can then be exploited in the development of new methods for tick population suppression.

2. How serious is the problem? Why does it matter?

The principal disease caused by deer ticks is Lyme disease. Lyme disease is the most prevalent vector-borne disease affecting humans in the U.S. with over 12,000 cases reported annually by the CDC. Lyme disease also seriously affects dogs and there is evidence to suggest that horses may also suffer from this disease. This same tick also transmits a newly discovered bacterial agent (Ehrlichia) closely related or identical to the agent which causes tick fever in sheep and cattle in Europe. This agent has been recently found to infect humans on both continents causing a disease known as human granulocytic ehrlichiosis. Lyme disease is already a serious health threat to humans in the U.S. and this new agent is potentially a serious health threat to both humans and domestic animals.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This project relates to two specific problems addressed in ARS National Program 104, Animal Pests and Parasites: 1) Environmentally safe methods

for control of pests and parasites, and 2) Tools for integrated management of animal/human pests and parasites. It shares the objectives of developing improved knowledge of the interrelations of ticks and vertebrate hosts to devise appropriate technologies for control, and developing efficient, cost-effective biologically-based control strategies for combating animal parasites. It specifically addresses the Program Component on ticks and mites by focusing entirely on the development of novel control methods for ticks.

4. What was your most significant accomplishment this past year?

Accession: 0400643 Year: 98 Project Number: 1265-32000-052-02 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 100%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

The most significant accomplishment of the past year was to determine the role of the deer tick as a vector of human granulocytic ehrlichiosis. We found the deer tick to be the only vector of this agent in the eastern U.S. We also found this agent to be transmitted by the deer tick completely independent of the agent of Lyme disease. These findings are significant in that they demonstrate the importance of the deer tick as a vector of diseases other than Lyme disease and emphasize the need for developing effective methods for population suppression of this tick species.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

We have determined that the most promising strategy for controlling deer tick populations in nature is to direct control efforts at the adult stage. This finding has already had impact on the design of new deer tick control programs by directing the focus of control efforts upon deer rather than hosts for the immature stages of ticks. We have also developed quantitative methods for estimating absolute density of deer ticks in the environment and for determining the prevalence of pathogens in these ticks. These two components constitute a direct measurement of the risk of infection for humans and animals. Accuracy in these methods will be essential in order to assess the efficacy of population suppression programs. In addition, we have initiated laboratory and field studies on the agent of human granulocytic ehrlichiosis in an attempt to determine its significance as a health threat to both humans and animals.

6. What do you expect to accomplish during the next year?

We expect to determine the relationship between deer tick density and the prevalence of tick-borne pathogens within the tick population. These relationships will be important in determining the levels of population suppression necessary to reduce transmission risk to humans and animals. Both field and laboratory studies will be conducted to determine these

relationships.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

All information produced by this project is directly applied to the USDA Northeast Regional Deer Tick Control Program. Demonstration of the importance of the deer tick as sole vector of human granulocytic

Accession: 0400643 Year: 98 Project Number: 1265-32000-052-02 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 100%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

ehrlichiosis in the eastern U.S. has resulted in the inclusion of this agent as principal object of study, in addition to the agent of Lyme disease. This information is now also available to other scientists and public health agencies to evaluate the total health impact of deer tick control programs.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

DESVIGNES, F. 1998. Transmission of the agent of human granulocytic ehrlichiosis. Thesis. Yale Univ. p. 103.

PUBLICATIONS:
01.
LEVIN, M.L. and FISH, D. 1998. Density-dependent factors regulating feeding success of Ixodes scapularis larvae (Acari: Ixodidae). J. Parasitol. 84:36-43.

02.
LEVIN, M.L. and FISH, D. 1998. Protection against reinfection with the agent of human granulocytic ehrlichiosis in immune white-footed mice. Amer. Soc. Trop. Med. Hyg. (Suppl.)59:279-280.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401252 Year: 98 Project Number: 1265-32000-052-03 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 100%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

Title: USDA NORTHEAST REGIONAL LYME TICK CONTROL PROJECT

Period Covered From: 08/97 To: 08/02

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The problem this project addresses is the expanding population of deer ticks in the U.S. which transmit Lyme disease, ehrlichiosis, and other diseases to both humans and domestic animals. This project is intended to resolve this issue by demonstrating the effectiveness of a novel control method designed to suppress populations of deer ticks at six study sites located in Lyme disease endemic areas of the northeastern U.S. The method uses a USDA-patented device that enables the self-application of tick-specific killing agents directly to wild deer, the principal reproductive host for the deer tick.

2. How serious is the problem? Why does it matter?

The principal diseases caused by deer ticks are Lyme disease and ehrlichiosis. Lyme disease is the most prevalent vector-borne disease affecting humans in the U.S. with over 12,000 cases reported annually by the CDC. Lyme disease also seriously affects dogs and there is evidence to suggest that horses may also suffer from this disease. This same tick also transmits a newly discovered bacterial agent (Ehrlichia) closely related or identical to the agent which causes tick fever in sheep and cattle in Europe. This agent has been recently found to infect humans on both continents causing a disease known as human granulocytic ehrlichiosis. Lyme disease is already a serious health threat to humans in the U.S. and this new agent is potentially a serious health threat to both humans and domestic animals. While a vaccine for Lyme disease has been recently developed, it is not approved for use by children and is marginally effective for persons older than 50. There are no vaccines

for ehrlichiosis or other diseases transmitted by the deer tick. There are no acceptable control methods for large-scale population suppression of deer ticks in disease endemic areas.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This project relates to two specific problems addressed in ARS National Program 104, Animal Pests and Parasites: 1) Environmentally safe methods for control of pests and parasites, and 2) Tools for integrated

Accession: 0401252 Year: 98 Project Number: 1265-32000-052-03 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 100%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

management of animal/human pests and parasites. It shares the objective of developing efficient, cost-effective biologically-based control strategies for combating animal parasites. It specifically addresses the Program Component on ticks and mites by focusing entirely on the implementation of a novel control method for ticks.

4. What was your most significant accomplishment this past year?

During the past year we have established base-line data on the density and infection prevalence of disease agents in the tick populations at each of the study sites. These initial data are essential for determining the effectiveness of the control program. We have also made adjustments to the protocol and to the self-application devices in order to improve effectiveness. Additionally, have determined that the density of deer at the study sites is significantly greater than original estimates, which have required additional adjustments, such as repositioning and increased maintenance of devices, and increased consumption of materials.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

The implementation of this demonstration project at six sites within five states where Lyme disease is hyperendemic is our most significant accomplishment to data. We now have more than 120 prototype USDA-patented self-application devices operative in the Northeast. Most of these are located on private property in suburban residential areas where the risk of Lyme disease to humans is most acute. This is the second year for the project, which is designed to run five years. Because of the two-year life cycle of the deer tick, measurable reduction of the tick population is not expected until year three or four. The predicted impact of this project is to bring about a significant reduction in the risk of Lyme disease and other deer tick-borne diseases within the study areas.

6. What do you expect to accomplish during the next year?

We will continue to maintain control pressure on the adult tick population through the spring and fall activity seasons during 1999. We expect to be able to determine a reduction in the density of infected ticks between the treatment and control areas within the study sites when we sample nymphal-stage ticks during the summer. The results of this assessment and the data gathered over the treatment period will be used to evaluate progress and make adjustments to the protocol as necessary.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401252 Year: 98 Project Number: 1265-32000-052-03 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.4 100%
NATL PROG(S) 104 Arthopod Pests of Animals & Humans 100%

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The technology resulting from this project should be available to public health agencies and municipal vector control programs by 2002. We know of no specific constraints that would prevent the adoption of this method of area-wide tick control among entities concerned about the risk of tick-borne disease.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401018 Year: 98 Project Number: 1265-32000-053-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 80% 3.2.2.1 20%
NATL PROG(S) 103 Animal Health 100%

Title: BIOCHEMICAL & MOLECULAR CHARACTERIZATION OF ENZYMES & BIOMOLECULES IN NEMATODE PARASITES

Period Covered From: 08/96 To: 08/01

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Grazing livestock acquire nematode parasites which cause losses in productivity due to decreased feed efficiency, morbidity and mortality. Control of nematode parasites relies entirely on the periodic application of anthelmintics. Although the efficacy of many of these drugs is high, continual treatment is required when transmission is ongoing. In some cases, drugs are only effective against some of the life cycle stages, leaving residual parasite populations. Further, many parasite populations have become resistant to one or more anthelmintic drug classes requiring use of higher doses or more expensive compounds. Developing novel control strategies to replace or supplement the use of anthelmintics requires an understanding of the biochemical mechanisms regulating key aspects of parasite development, nutrition and host interactions. The description of enzyme systems and other functional or structural elements of these parasites will facilitate the design of targeted drug and immunological controls. Taeniasis (Taenia saginata) is one of the major food-borne parasitic diseases in the U.S. The disease is acquired by the ingestion of raw or rare beef infected with the larval stage of this parasite (beef cysticercosis). Elimination of the disease relies on meat inspection procedures which are labor-intensive and highly inaccurate. There is a need to develop a sensitive and specific serology assay for bovine cysticercosis.

2. How serious is the problem? Why does it matter?

Costs to producers resulting from nematode parasitism include mortality, morbidity, additional veterinary care and, most importantly,

expenditures for anthelmintic treatment. Estimates suggest total industry losses of approximately $800 million annually due to nematode parasites of ruminant livestock. Bovine cysticercosis remains a public health threat and the specific inspection of all cattle at slaughter for this parasite is mandated. A reliable serology test for cysticercosis could replace or supplement the current labor-intensive and unreliable physical detection procedure thus ensuring a safer food supply and controlling the spread of this disease to humans. The test could be used to screen all animals at the farm or feedlot in high incidence regions to identify infected animals before they reach the slaughterhouse; this

Accession: 0401018 Year: 98 Project Number: 1265-32000-053-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 80% 3.2.2.1 20%
NATL PROG(S) 103 Animal Health 100%

information would also help in establishing specific point sources of infection. The test could also be used to screen animals imported from high incidence countries such as Mexico, eliminating the influx of infected animals.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research contributes to disease prevention and control (National Program 103 Animal diseases) by describing critical new fundamental information on the biology of parasites that provides a rationale for development of novel control strategies to reduce losses caused by parasitic diseases of livestock. The research also supports program objectives of developing better tools for detecting zoonootic pathogens in food animals.

4. What was your most significant accomplishment this past year?

We developed a rodent model to evaluate the efficacy of synthetic protease inhibitors on development of the stomach worm Haemonchus contortus. Synthetic peptides designed as specific inhibitors of cysteine proteases were obtained from collaborators at Enzyme Systems, Inc., and were evaluated for efficacy and toxicity in the rodent model. These studies demonstrate the potential of targeted enzyme inhibition for control of nematode parasites.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Cysteine proteases in the stomach worm Haemonchus contortus were identified and characterized as potential targets for control methods. Synthetic peptide inhibitors previously developed for biomedical purposes were shown to be potent inhibitors of the parasite proteases. We characterized, purified, sequenced and cloned a hemoglobin-like protein from Haemonchus contortus. As a major constituent of the soluble

protein fraction of the parasite, this protein plays a role in adapting the parasite to the host by its ability to transport oxygen, and serve as an iron reservoir and osmoregulator. Novel enzyme inhibitors in secretory products of Trichuris suis, the pig whipworm, were purified, characterized and sequenced. These unique peptide inhibitors may function as a parasite defense mechanism for evasion of host immunity, by down-regulating effector cell mechanisms and may be an important contributing factor to the more severe clinical aspects of T. suis infections. In addition to their importance for parasite survival, the unique properties of parasite protease inhibitors are now recognized for

Accession: 0401018 Year: 98 Project Number: 1265-32000-053-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 80% 3.2.2.1 20%
NATL PROG(S) 103 Animal Health 100%

their potential benefits in the treatment of human diseases. The unique peptide inhibitors of T.suis may also have potential in the treatment of various human pathologies including cancer and inflammatory diseases. We characterized enzymes from excretory/secretory products of adult and larval stages of nematode parasites, including Haemonchus contortus, Trichuris suis and Ascaris suum. These enzymes include proteolytic enzymes and phosphatases that play significant roles in parasite nutrition, development, molting and in adaptation to the host environment. We also characterized and localized a hemolytic factor from H. contortus that plays a key role in breaking host red blood cells to provide access to nutrients essential for parasite survival. We identified and purified a sensitive and specific antigenic reagent for the diagnosis of bovine cysticercosis. Over 800 sera from cysticercosis-suspect cattle at feedlots in Colorado, Idaho, and California were evaluated using a potential cysticercosis-specific antigen (ThFAS) identified in our laboratory. Information from serological studies was provided to producers and used in making slaughter decisions.

6. What do you expect to accomplish during the next year?

We will complete studies on the effect of synthetic protease inhibitors on development of H. contortus in the rodent model and complete characterization and purification of novel protease inhibitors and evaluate their effect on host immune and inflammatory cell reactions.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Collaboration was formed with an industry, Enzyme Systems, Inc., group to evaluate the effects of a number of synthetic cysteine protease inhibitors on Haemonchus contortus infection in an animal model. Elimination of a nematode from its host by administration of a specific protease inhibitor would give considerable impetus to current research

on nematode proteases and other enzymes involved in maintaining host-parasite relationships and to efforts by the pharmaceutical industry to produce effective, non-toxic inhibitors. Completion of these studies will allow transfer of this important information to the pharmaceutical industry. A limitation to this technology is the development of effective inhibitors that have acceptable toxicity levels. Antemortem testing for the presence of cysticercosis in cattle at feedlots using the diagnostic test developed in our laboratory provided vital information to feedlot managers concerning the scope and source of the infection and in decisions concerning time of slaughter.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401018 Year: 98 Project Number: 1265-32000-053-00 D
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 80% 3.2.2.1 20%
NATL PROG(S) 103 Animal Health 100%

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

01.
FETTERER, R.H. and RHOADS, M.L. 1997. The in vitro uptake of albumin by adult Haemonchus contortus is altered by extra-corporeal digestion. Vet. Parasitol. 73:249-246.

02.
FETTERER, R.H., RHOADS, M.L. and HILL, D.E. 1998. A hemolytic factor from Haemonchus contortus alters erythrocyte morphology. Vet. Parasitol. 80:37-45.

03.
FETTERER, R.H. and HILL, D.E. and RHOADS, M.L. 1998. Characterization ... Haemonchus contortus. Proceedings of 43rd Ann. Mtg. Am. Soc. Parasitol. July, 1998, Baltimore, MD. p. 40.

04.
RHOADS, M.L and FETTERER, R.H. 1998. Purification and characterization of a secreted aminopeptidase from adult Ascaris suum. Int. J. Parasitol. 28:1681-1690.

05.
RHOADS, M.L., FETTERER, R.H. and URBAN, J.F., Jr. 1998. Effect of protease class-specific inhibitors on in vitro development of ... larvae of Ascaris suum. J. Parasitol. 84:686-690.

06.
LU, C.C., NGUYEN, T. MORRIS, S., HILL, D.E. and SAKANARI, J.A. 1998. Anisakis simplex: Mutational bursts ... serine protease inhibitors from

ascarid nematodes. Exp. Parasitol. 89:257-261.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0148348 Year: 98 Project Number: 1265-32000-053-01 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 100%

Title: FIELD TESTING OF ELISA FOR THE SERODIAGNOSIS OF SWINE TRICHINELLOSIS

Period Covered From: 01/98 To: 10/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Trichinella spiralis is a nematode parasite that infects a wide variety of species, including humans. The major agricultural issue with respect to T. spiralis is food safety; Trichinella infection results from eating infected meat, primarily pork products. Consumers have long been aware of the potential presence of T. spiralis in pork and the fact that it poses a risk to those who eat raw/undercooked or otherwise improperly prepared meat. The objectives of ARS research conducted on T. spiralis includes reducing or eliminating risk of human exposure from contaminated pork or other meats products. A major component of these efforts is the availability of a sensitive and specific test which can detect Trichinella infection in pigs. In this CRADA, ARS and industry cooperators developed detection systems for Trichinella in pigs.

2. How serious is the problem? Why does it matter?

Trichinella spiralis is the #1 consumer concern with respect to the safety of pork products. The U.S. requires extensive processing of all ready-to-eat pork products. Trading partners require testing of pork products prior to shipment, and the image of U.S. pork with respect to Trichinella infection impacts accessability to foreign markets. As the pork industry moves to develop certification programs which document product safety, it is necessary to have tools available which can accurately detect infection in pigs.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research conducted on this project supports National Program objectives in Animal Health and Food Safety (National Program 103, Animal Diseases and National Program 108, Food Safety). Objectives of research on the zoonotic pathogen Trichinella focus on detection, prevalence, transmission, and epidemiology. The goal of this research is to establish farm management systems (pre-harvest interventions) which limit or eliminate risk of infection in food animals. Accurate testing systems are needed to identify infected animals.

4. What was your most significant accomplishment this past year?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0148348 Year: 98 Project Number: 1265-32000-053-01 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 100%

A sensitive and specific diagnostic test was developed. This test has flexibility in that it can be conducted using blood, serum or tissue fluids. Further it can be adapted to run as a lateral flow system, allowing the test to stay with the animal during processing. Question 5: A highly sensitive and specific test system (ELISA) was developed to detect Trichinella infection in pigs. Using conventional excretory-secretory (ES) antigens, this test was validated using blood, serum and tissue fluids as test samples. The ES antigens were compared with a synthetic gylcan antigen in ELISA using several hundred sera from experimentally infected pigs and over 100 sera from pigs with naturally acquired infection (obtained from Romania). These results indicated that the gylcan antigen was also acceptable for use in the detection of Trichinella infection in pigs. To allow flexibility in the use of the test, it was placed into a lateral flow format. This system, which can be carried with the carcass during processing, was as effective as ELISA in detection of infection in preliminary studies.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

6. What do you expect to accomplish during the next year?

This CRADA terminated 31 October, 1998 and no further work is planned.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The ELISA test for Trichinella infection is pigs is available commercially from Safe-Path Laboratories, Minneapolis, MN and has been used extensively by state and federal diagnostic labs. This test will be used as a means of verification in an industry wide certification program for trichinae in pigs. Manuscripts describing this work were

reported in previous years.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0148728 Year: 98 Project Number: 1265-32000-053-02 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.2 100%
NATL PROG(S) 103 Animal Health 100%

Title: CHARACTERIZATION OF NEMATODE EXCRETORY-SECRETORY PRODUCTS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Nematodes are ubiquitous internal parasites of domestic livestock and have evolved into highly specialized organisms that produce biomolecules having significant impact on host tissues. Collection and characterization of such molecules can provide not only targets for the development of new control strategies but also novel compounds that may be uniquely suited for critical therapeutic functions in the biomedical sciences. This project is a collaborative effort between ARS and BioPharm, Inc. to study and exploit parasite products.

2. How serious is the problem? Why does it matter?

Costs to producers resulting from nematode parasitism include mortality, morbidity, additional veterinary care and, most importantly, expenditures for anthelmintic treatment. Estimates suggest total industry losses of approximately $800 million annually due to nematode parasites of ruminant livestock. Control of nematode parasites relies entirely on the periodic application of anthelmintics. Treatment regimens vary, but the most effective use of drugs generally targets peak periods of parasite transmission, particularly transmission to young animals. Although efficacy of many drugs is high, continual treatment is required when transmission is ongoing. In some cases, drugs are only effective against some of the life cycle stages, leaving residual parasite populations. Further, many parasite populations have become resistant to one or more anthelmintic drug classes requiring use of higher doses or more expensive compounds.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research contributes to disease prevention and control (National Program 103 Animal Diseases) by providing new information that can be used to develop novel control strategies to reduce losses caused by nematode diseases of livestock.

4. What was your most significant accomplishment this past year?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0148728 Year: 98 Project Number: 1265-32000-053-02 T
Mode Code: 1265-40-00 STP Codes: 3.2.2.2 100%
NATL PROG(S) 103 Animal Health 100%

We identified and characterized protease inhibitors in the swine whipworm Trichuris suis and characterized phosphatase enzymes in adult and larval stages of Haemonchus contortus.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

We developed methods for the in vitro culture and maintenance of the livestock nematodes, Ascaris suum, Haemonchus contortus and Trichuris suis and the collection and characterization of the excretory/secretory products from these parasites.

6. What do you expect to accomplish during the next year?

We will characterize protease inhibitors from Trichuris suis including amino acid sequencing and cloning of at least one of the inhibitors.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The methods for cultivation and collection of nematodes were made available to collaborators at BioPharm, Inc., through direct communication and publications. Excretory/secretory products from nematodes were supplied to collaborators for identification of various activities including anticoagulant, platelet aggregation inhibition and anti-inflammatory activity.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149861 Year: 98 Project Number: 1265-32000-053-03 R
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 70% 3.2.2.5 30%
NATL PROG(S) 103 Animal Health 100%

Title: CHECK SAMPLE PROGRAM FOR TESTING HORSEMEAT FOR TRICHINAE

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Trichinella spiralis is a nematode parasite that infects a wide variety of species, including humans. The major agricultural issue with respect to T. spiralis is food safety; Trichinella infection results from eating infected meat, primarily pork products. Consumers have long been aware of the potential presence of T. spiralis in pork and the fact that it poses a risk to those who eat raw/undercooked or otherwise improperly prepared meat. Because the U.S. has never inspected its pigs for Trichinella, infection rates in pigs have not declined as precipitously as in countries where inspection is routinely practiced. European Union and Russian export markets require the U.S. to test pork and horsemeat for Trichinella prior to shipment. This project supports a cooperative program with the Agricultural Marketing Service in which ARS provides training and support to an inspection program in U.S. slaughter plants.

2. How serious is the problem? Why does it matter?

Trichinella spiralis is the #1 consumer concern with respect to the safety of pork products. The U.S. requires extensive processing of all ready-to-eat pork products. Trading partners require testing of pork products prior to shipment, and the image of U.S. pork with respect to Trichinella infection impacts accessability to foreign markets. Without this project, the U.S. could not ship pork or horsemeat to Europe and Russia.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research conducted on this project supports National Program objectives in Animal Health and Food Safety (National Program 103, Animal Diseases and National Program 108, Food Safety). This work supports inspection testing methodology which is integral to maintenance of export markets.

4. What was your most significant accomplishment this past year?

Verified critical control points in the pooled sample digestion method

Accession: 0149861 Year: 98 Project Number: 1265-32000-053-03 R
Mode Code: 1265-40-00 STP Codes: 3.2.1.4 70% 3.2.2.5 30%
NATL PROG(S) 103 Animal Health 100%

in support of methods used to test pigs and horses for Trichinella. These results provide technical support of U.S. inspection methods which allow export of pork and horse meat to EU countries and Russia.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

This project provides for training of approximately 20-25 meat inspectors each year. In addition, a quality assurance program is conducted on a quarterly basis to maintain the integrity of the testing program. Several experiments have been conducted to validate aspects of the pooled sample digestion testing methods which are used to inspect for Trichinella in pigs and horses. The results of these studies are used to support the methods used by U.S. meat inspectors in negotiations with EU and Russian agriculture officials.

6. What do you expect to accomplish during the next year?

This agreement with the Agricultural Marketing Service is renewed each year and will continue as long as requirements are in place for testing exported pork and horse meat for Trichinella. As needed, experiments will be conducted to validate inspection methods.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The training provided to pork and horse meat industry inspectors is integral in their participation in the export program. New methodology and information is included into this training when appropriate. When necessary, the ARS scientist conducts research to resolve disputes on inspection methodology. As needed, the ARS scientist on this project represents technical aspects of the program to agricultural officials from importing countries.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401126 Year: 98 Project Number: 1265-32000-053-04 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 100%

Title: CYSTEINE PROTEASE INHIBITORS AS CONTROL AGENTS FOR HAEMONCHOSIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Nematodes, such as the sheep stomach worm Haemonchus contortus, are ubiquitous internal parasites of domestic livestock. Recent results from this laboratory have demonstrated that cysteine proteases are present in the worm and its secretions and these enzymes play a key role in adapting the parasite to the host. Inhibition of this enzyme has been identified as a potential novel method of control.

2. How serious is the problem? Why does it matter?

Costs to producers resulting from nematode parasitism include mortality, morbidity, additional veterinary care and, most importantly, expenditures for anthelmintic treatment. Estimates suggest total industry losses of approximately $800 million annually due to nematode parasites of ruminant livestock. Control of nematode parasites relies entirely on the periodic application of anthelmintics. Treatment regimens vary, but the most effective use of drugs generally targets peak periods of parasite transmission, particularly transmission to young animals. Although efficacy of many drugs is high, continual treatment is required when transmission is ongoing. In some cases, drugs are only effective against some of the life cycle stages, leaving residual parasite populations. Further, many parasite populations have become resistant to one or more anthelmintic drug classes requiring use of higher doses or more expensive compounds.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research contributes to disease prevention and control (National Program 103 Animal diseases) by developing new information that can be used to develop novel control strategies to reduce losses caused by nematode diseases of livestock.

4. What was your most significant accomplishment this past year?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401126 Year: 98 Project Number: 1265-32000-053-04 S
Mode Code: 1265-40-00 STP Codes: 3.2.2.1 100%
NATL PROG(S) 103 Animal Health 100%

We developed a rodent model to evaluate the efficacy of synthetic protease inhibitors on development of the stomach worm Haemonchus contortus. Synthetic peptides protease inhibitors designed as specific inhibitors of cysteine proteases were obtained from collaborators at Enzyme Systems, Inc. and were evaluated for efficacy and toxicity in this model.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Cysteine proteases from the stomach worm Haemonchus contortus were identified and characterized as potential targets for control. Synthetic peptide inhibitors previously developed for biomedical purposes were shown to be potent inhibitors of the parasite protease.

6. What do you expect to accomplish during the next year?

We will complete studies on the effect of synthetic protease inhibitors in vivo on development of H. contortus.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Collaboration was formed with an industry group at Enzyme Systems, Inc. to evaluate the effects of a number of synthetic cysteine protease inhibitors in an animal model. Completion of these studies will allow transfer of information to the pharmaceutical industry. Limitations to this technology include developing effective inhibitors that have acceptable toxicity levels in animals.

8. List your most important publications and presentations, and articles

written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL